Understanding attraction stimuli of Ctenocephalides felis for non-chemical control methods

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Understanding attraction stimuli of *Ctenocephalides felis* for non-chemical control methods.

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Abstract. Comparisons of flea catches by four commercially available flea traps were conducted in the laboratory and under field conditions, in both rural and urban locations. The results clearly showed My Flea Trap, which utilizes an intermittent light for attracting fleas, to be far superior in trapping ability than the three continuous light traps, catching up to 23 fold more fleas than the other traps. Alteration of the lighting mechanism from intermittent light to continuous light significantly decreased the numbers of fleas captured. In addition, the use of a green filter significantly increased the trapping efficiency, while the addition of a heat source had no apparent effect.

Key Words. *Ctenocephalides felis***,** cat fleas, attraction-stimuli, non-chemical control, traps

Introduction

The hematophagous insect *Ctenocephalides felis felis*, the cat flea, is the prevailing ectoparasite found on cats and dogs (reviewed in Rust & Dryden 1997, Rust

2005). Besides causing major discomfort for both pets and pet-owners, *C. felis* has been found to be the main elicitor of flea allergic dermatitis (FAD) (Schiedt 1998), and severe infestation can lead to iron deficiency anemia, a potentially lethal affliction in young animals (Yeruham *et al.* 1989, Dryden *et al.* 1993). *C. felis* can also act as a vector for several pathogens and parasites, including *Rickettsia felis*, *Rickettsia typhi*, the cause of murine typhus (Traub *et.al.* 1978, Azad 1990), *Bartonella henselae* (Breitschwerdt 2008), *Mycoplasma haemofelis* (Woods *et al.* 2005) and *Dipylidium caninum,* an intestinal cestode which parasitizes dogs, cats, and occasionally, children (Chen 1934, Molina *et al.* 2002). Considered to be a frequent annoyance to domestic pet owners worldwide, the global expenditure on flea-control products has increased over the last decade to over 2 billion $US a year (Coniff 1995, Krämer & Mencke 2001, Rust 2005).

Chemical control agents given topically or orally have been shown to be very effective in eliminating flea infestations (Reviewed in Carlotti & Jacobs 2000). Insecticides such as fipronil and imidacloprid have been shown to be >95% effective in killing the adult population, as well as the off-host populations for periods longer then 28 days (Ritzhaupt *et al.* 2000, McTier *et al.* 2003). Other chemical formulations, such as juvenile-hormone analogues (JHA's) and insect developmental inhibitors (IDI's) are also very effective in preventing infestations and re-infestations (Shipstone & Mason 1995) of these pests. Despite the advances in chemical formulations for flea control, re-infestations are still a common occurrence. Although most of these are a result of owner non-compliance with application instructions, resistance of flea populations to several agents has been shown to occur, including permethrin (Bossard *et al.* 2002, Lemke *et al*. 1989), other pyrethroids, and organo-phosphates (Bossard *et al.* 1998). Furthermore, the increased use of newer classes of insecticides which are available on the market is likely to induce selection pressure on flea populations which may lead to resistance to these compounds (reviewed in Sangster 2001).

In addition to the aforementioned resistance potential, one of the major problems in combating fleas is that often by the time pet owners are aware of fleas on their pets there is already an infestation of the premises by immature flea life stages. Therefore, following initiation of chemical treatments to the pets or even the premises, fleas will continue to develop and emerge. These fleas can reinfest pets and bite humans in the home for several weeks causing great discomfort (Dryden 2009).

Additionally, environmental and toxicological concerns have been raised in the last few years at the increased use of chemical insecticides for controlling flea infestations. Environmental issues include toxicity to non-target insects (Overmyer *et al.* 2005), accumulation in the environment, and possible toxicity to pets and people prone to higher exposure to the flea agents (Ames *et al.* 1989, Fritschi 2000). Although the latter are attributed mainly to older-generation insecticides such as organophosphates and organochlorides (reviewed by Hovda *et al.* 2000), the adverse possibilities have spurred motivation to develop efficient flea-traps that can match the efficiency of the chemical control agents sold on the market. Since fleas use both thermal and visual cues for targeting potential hosts, most traps available on the market use incandescent light bulbs to generate these attracting stimuli. A thorough investigation of the optimal lighting spectrum required to attract *C. felis* was conducted by Dryden and Broce (1993), in which several light filters, as well as light source positions, and light regime were tested. The study found that the optimal spectrum for attraction of *C. felis* was yellow-green light

(510-550 nm), and that periodic interruption of the light source dramatically increased the trapping efficiency. These results have been the foundation for development of the commercially available intermittent light trap (ILT), called My Flea Trap. As results under laboratory conditions can vary significantly from those obtained under field conditions, this study also compared commercially available traps in field trials.

Materials and methods

Tested traps

Trap comparisons were conducted using four different commercially available flea traps which were bought through the internet (Fig 1 A-D). All four traps that were compared use light as the attracting stimulus, although of varying spectra and positions above the ground. Additionally, all traps utilize glue paper for immobilizing the attracted insects. Descriptions of other trap attributes, as well as manufacturer specifications are as follows:

My Flea Trap (Westham innovations): Utilizes two narrow spectra light emitting diodes (LED) which operate in alternating sequence, both positioned 25 cm above the ground. The trap base is semicircular measuring 29 cm in diameter. The lights are powered by 4 AA batteries lasting 100,000 hours according to manufacturer claims printed on the packaging. Claims also include attraction of fleas from 40 feet and beyond, and clearance of the fleas from the infested room after one night of trap operation.

Ultimate Flea Trap (Victor): Utilizes a 7W type A light bulb suspended 10 cm above the ground over the round trap base (18 cm diameter) which houses the glue disc used to trap the attracted fleas. The trap operates using 110V AC for power supply. Manufacturer claims printed on the packaging include 93% catch rate, and attraction of fleas from a distance of 9 meters.

Springstar Flea Trap (previously known as NuPu): Utilizes a 7W type A bulb suspended 5 cm above the ground. Trap base is rectangular, 16 cm x 14 cm. The trap operates using 110V AC for power supply. Manufacturer claims the trap attracts fleas from 25 feet away under normal conditions.

Flea Beacon (Happy Jack Inc.): Utilizes a 7W type A bulb suspended 10 cm above the ground, and uses 110V AC power supply. Trap base is rectangular, 30 cm x 21 cm, with a top cover of identical dimensions.

Field trials

Trap comparisons in the field were conducted in both rural and urban locations. The rural site (Site I), is located in the lower Jordan Valley, Israel. Trials were conducted in late October 2006, in an 8 x 4 meter stable constructed of concrete and clay, housing various livestock, including goats, sheep, dogs and chickens, and with a substantial rodent infestation (mainly *Mus musculus* L. and *Rattus rattus* L.). Traps were placed in the four corners of the stable, close to the walls, and rotated clockwise every 24 hours for eight consecutive days, until all traps occupied each corner twice. At the end of each day the fleas were collected from the trap and counted.

Urban field trials (Site II) were conducted during November 2006, in four different chemically-untreated apartments south of Tel Aviv, Israel each housing a single cat. Traps were placed in the four corners of the living rooms of the different apartments, each measuring between 25 and 44 square meters. Traps were rotated clockwise every 24 hours and positioned in a different corner for four consecutive days in each apartment. Fleas were collected and counted every 24 hours, before rotation of the traps.

Laboratory trials

Two separate laboratory trials were conducted. The first laboratory traps comparisons were conducted in mid august, 2007, in a carpeted room measuring 4 x 6 meters, lit by ambient sunlight through three 0.7 m^2 windows. Fleas were collected with sweep nets at Site I and brought to the laboratory, where they were anesthetized with CO_2, divided into groups of 100, and sealed in jars which were kept on ice until the trials that were conducted the next day as follows: A jar containing 100 fleas and one of the tested traps were positioned in diagonal corners of the room 7.2 meters apart as described above. The fleas were released from the jar, and collected either after 24 hours or during the light hours (8:00-18:00). Dead fleas from the jar and trapped fleas were collected and counted, and surviving fleas were removed from the carpet with a vacuum cleaner at the end of each repetition. Trials were conducted eight times for each trap tested.

The second set of laboratory trials were conducted at Kansas State University, Manhattan KS, USA, also in August 2007, and compared My Flea Trap (Westham innovations), against Springstar Flea Trap (previously known as NuPu), Ultimate Flea trap (Victor) and Flea Beacon (Happy Jack Inc.). Traps were tested in two rooms, with traps randomly allocated to each room. The rooms measured 2.1 x 2.4 m. The traps were centered against the wall and 100 *C. felis* (KS-1 Laboratory Colony), approximately 3 days post emergence, were placed 2.4 m across the room from the trap. The rooms were completely dark except for the light of the traps. The floors were vacuumed between trials to remove any remaining fleas. For trap comparisons, the My Flea Trap was tested against each of the other two traps in separate trials. For each trap comparison a trial was conducted using a trapping interval of 7 – 8 hr and a separate trial evaluating flea collections at 15 – 16 hr. Trials were conducted three times for each trap and trapping interval tested.

Comparison of modified traps

To assess the importance of the lighting mechanism on the performance of the most efficient flea trap, the lighting sequence of the intermittent light trap (My Flea Trap) was modified and compared to the best performing continuous light trap (Ultimate Flea Trap) under field conditions. The modification of the My Flea Trap consisted of changing the lighting pattern from synchronous flickering of the two light sources to either alternating flickering or continuous light. Trials were conducted at Site I in early June, 2007, following a pesticide treatment of the site in early April, 2006 which significantly reduced flea population numbers. Traps were placed at the four corners of the stable and rotated every 24 hours for 8 consecutive days, until all traps occupied each corner twice. Fleas were collected from the traps and counted at the end of each day.

Additional trials were conducted to assess the consequence of adding a heat stimulus on trapping efficiency, and the significance of using a narrow bandwidth 525 nm light source. Both My Flea Trap and Ultimate Flea Trap were equipped with a heat source consisting of a 7W light bulb placed in a tight fitting plastic tube and sealed with black electrical tape to block any light. The heat sources were placed below the light source of each trap. In addition, an Ultimate Flea trap was modified by placing a green filter on the light source, as described by Broce & Dryden (1993). Comparisons of the traps were conducted at Site I from early to mid-September, 2007. The modified traps were placed in the four corners of the stable, and rotated clockwise every 24 hours after collection of fleas, for a total of nine repetitions for each trap.

Statistical analysis

Analysis of variance (ANOVA), as well as student t-tests were performed to check for statistical differences among the efficiencies of the four traps. Differences between groups were performed using Tukey's post hoc test. All statistical tests were performed with freeware available on the internet through Kirkman, T.W. (1996) "Statistics to Use". http://www.physics.csbsju.edu/stats/ (accessed January 23, 2009: April 30, 2009)

Results

Trap comparisons in field and laboratory trials

In the rural field trials (Fig. 2), the number of fleas caught during the eight day period (Mean ± SE = 1023.12 ±171.77, n=8) was significantly higher for the My Flea Trap than for the three other traps tested (9.8, 12.4 and 6.7 fold more than Springstar, Ultimate Flea Trap, and Flea Beacon, respectively, ANOVA F = 26.59, dF=3,28, $P <$ 0.01 Tukey test) . No significant difference was seen between Springstar, Ultimate Flea trap, and Flea Beacon (Mean ± SE = 103.87 ± 18.21, 151.87 ± 31.14, and 82.25 ± 22.25, respectively). The same trend was observed under urban field trials (Fig. 3), in which My Flea trap attracted 7 – 23 times more fleas than the other traps (ANOVA F = 2.91, dF=3,60, $P < 0.01$ Tukey test), although these figures mainly reflect the relatively low number of fleas caught in the urban field trials (Mean ± SE = 8.56 ± 1.4, 0.9 ± 0.2, 1.25 ± 0.26 and 0.375 ± 0.15 fleas caught by My Flea trap, Springstar, Ultimate flea trap, and Flea Beacon, respectively, n=16). A significant difference was also seen between flea captured by the Ultimate Flea Trap and the Flea Beacon Trap (t-test, P< 0.05).

The results from the laboratory trials conducted in Israel (Fig. 4) are consistent with those seen in the field trials. When collected after 24 hours, My Flea Trap trapped almost all the fleas released into the rooms (Mean ± SE = 92.65 ± 1.93 percent, n=8), which was significantly higher when compared to the relatively low numbers trapped by the Springstar, Ultimate Flea trap, and Flea Beacon (ANOVA F = 350.47, dF = 3, 28, P < 0.01 Tukey test). No significant differences were seen among the fleas trapped, as mentioned above (Mean ± SE = 13.18 ± 1.9, 16.46 ± 2.8, and 8.26 ± 1.8 percent, respectively). Likewise, fleas collected at the end of the daylight hours yielded similar results, with My Flea Trap attracting significantly more fleas than the other traps (ANOVA F = 263.46, dF = 3, 28, P < 0.01 Tukey test).

All traps attracted significantly more fleas when operated for 24 hours, compared to when operated only during the daylight hours (*t* -test, P < 0.01, Fig. 3 gray and black bars). The increase was more prominent in the three continuous light traps, mainly due to the low numbers of trapped fleas throughout both experiments. Nevertheless, the three continuous traps attracted more fleas during the night, as indicated by the relatively low numbers of fleas caught during the daylight hours (Mean ± SE = 2.95 ± 0.8, 4.24 ± 0.72, 1.77 ± 0.4 fleas, Springstar, Ultimate Flea Trap and Flea Beacon, respectively, n=8) compared to the whole 24 hour period (Springstar-13.18 ± 1.14, Ultimate Flea Trap -

16.46 ± 2.8, Flea Beacon- 8.27 ± 1.8, n=8). In contrast, My Flea trap attracted a high percentage of the released fleas during the daylight hours (71.3 ± 4.3 percent, n=8), with ca. 20 percent more caught on the 24 hour trapping trials (92.65 ± 1.93 percent, n=8).

In the paired laboratory trials conducted at KSU, the My Flea Trap collected more fleas than the Springstar trap at 7 – 8 hr (*t* -test, P < 0.01) and 15 – 16 hr (*t* -test, P < 0.01). The My Flea Trap and the Springstar trap collected a mean of 55.0 and 19.3 fleas and 66.0 and 28.0 fleas, respectively at 7 – 8 hr and 15 – 16 hr. In the second set of trap comparisons at KSU, the My Flea Trap collected more fleas than the Ultimate Trap at 7 – 8 hr (*t* -test, P = 0.011) but did not collect statistically more fleas at 15 – 16 hr (*t* -test, P = 0.190). The My Flea Trap and the Ultimate Trap collected a means of 41.7 and 19.1 fleas and 52.3 and 31.3 fleas, respectively at 7 – 8 hr and 15 – 16 hr. In the third set of trap comparisons at KSU the My Flea trap did not collect statistically collect more fleas than the Flea Beacon trap at 7 – 8 hr (*t* -test, P = 0.302) or at 15 – 16 hr (*t* -test, P = 0.549). My Flea trap and the Flea Beacon trap collected a mean of 41.7 and 19.1 fleas and 52.3 and 31.3 fleas, respectively at 7 – 8 hr and 15 – 16 hr.

Comparison of modified flea traps

The high trapping efficiency of the My Flea Trap can be attributed either to the spectrum and intensity of the attracting light (525 nm light emitting diode), or to the lighting regime (see discussion). For assessment of whether the intermittency of the light stimulus is the parameter responsible for the higher trapping efficiency of the My Flea Trap, the light regimes of two different My Flea Traps were modified and compared to a standard My Flea Trap, as well as an Ultimate Flea Trap, which showed the best results out of the three continuous light traps which were tested in the field trials. The results presented (Fig. 5) show that alteration of the light stimulus from synchronous to alternating flickering had caused a slight reduction in the numbers of fleas caught (149.9 ± 17.88, 103.5 ± 16, respectively), albeit not statistically significant. However, alteration of the ILT lighting from synchronous flickering to continuous light had resulted in a dramatic 5 fold decrease in the numbers of fleas which were trapped (ANOVA F = 26.59, dF = 3,44, $P < 0.01$ Tukey test). Nevertheless, the modified My Flea Trap utilizing continuous light still managed to trap significantly more fleas than the Ultimate flea trap (t-test, $P < 0.05$). The effects of the specific light spectrum used by My Flea Trap were evaluated in subsequent field trials, in which the light source of the Ultimate flea trap was modified by a green filter as described by Broce & Dryden & Brroce (1993). The results (Fig. 6) indicate that modification of the light with a green filter significantly improves trap efficiency (t-test, $P < 0.05$), catching 1.57 more fleas than the control trap which utilized the original polychromatic 7W light source (143.33 ± 16 and 91.9± 16.8, respectively).

Modifications of traps to include a heat source did not significantly enhance trapping ability. The results (Fig. 7) indicate that although a slight rise can be seen when adding a heat source to My Flea Trap (752.78 ± 59.1 with heat, 674.9 ±, 59.4 without heat), it is not statistically significant (t-test $P = 0.37$). In fact, the trap utilizing heat as the only attracting stimulus did not trap significantly more fleas than the control trap with no attracting stimulus (18.55 ± 3.5 and 11.1 ± 2.5, respectively).

Discussion

Despite the high efficiency of insecticides in controlling flea infestations, alternative control methods such as flea traps are gaining favor due to environmental and toxicity concerns, as well as their prospective use when individual treatment is difficult or impossible. Better understanding of the host seeking mechanisms of *C. felis* is a fundamental requirement for construction of an efficient flea trap, which could be utilized for sample collection in research studies, or to control infestations by breeders, animal shelters, as well as domestic-pet owners. The results described herein have further demonstrated the advantages of using an intermittent light source for trapping *C. felis*. In field evaluations the My Flea Trap consistently attracted and trapped more fleas than the other traps tested, which utilize a continuous polychromatic light source as the attracting cue (Fig. 2- 4). In the laboratory trials there were some differences between the trials conducted in Israel and those in the U.S. This may be due to differences such as characteristics of the different flea strains used in the evaluations and the age of fleas post-emergence. However, it is likely that the reason that there were not statistical differences between the My Flea trap and the Ultimate trap at 15 – 16 hr and the Springstar trap at either trapping period was the relatively short distance used in the evaluation. In the U.S. trials the distance between the fleas and traps was 4.2 m, whereas in the laboratory trials conducted in Israel the distance was 7.2m. The effect of distance is evidenced by the fact that during a trapping period of 7 – 8 hr, the My Flea Trap collected statistically more fleas that the Ultimate trap but not at 15 – 16 hr.

Additionally, as expected, each trap collected more fleas during the 15 – 16 hr period than at the 7 – 8 hr period. These data demonstrate that there is less difference in trapping rates of the flea traps in smaller areas, but a more efficient trap should collect more fleas more rapidly and should collect more fleas in larger rooms.

These results are in agreement with laboratory comparisons of continuous and intermittent light sources in which seven to eight times more fleas were trapped by the latter (Dryden and Broce 1993). The My Flea Trap, constructed in accordance with the observations in the above publication, utilizes two LED with a narrow spectrum which operates in an intermittent fashion for attracting fleas. The significance of the intermittency of the light was demonstrated through modification of the lighting regime from intermittent to continuous light, which dramatically reduced the trapping efficiency by 5 fold (Fig. 5). Nevertheless, other factors still account for the better attracting efficiency of the My Flea Trap, since the modified trap still caught significantly more fleas than the Ultimate light trap. To test whether the specific light spectrum is responsible in part for the better attracting abilities, subsequent comparisons were conducted between Ultimate Flea Traps with and without a green filter, in which the former trapped significantly more fleas (Fig. 6), ascertaining the notion that fleas are more attracted to 525 nm light.

The three continuous light traps tested use a 7W type A bulb for flea attraction, with specifications that this type of bulb provides both light and heat for attracting fleas. However, although fleas have been shown to use both visual and thermal cues in host seeking (Osbrink et al., 1985), addition of a heat source to My Flea trap did not significantly increase trapping efficiency (Fig. 7). Moreover, a trap using heat as the only attracting stimulus did not perform better than the control trap which used no attracting stimulus. The same results were obtained when adding the same heat source to the

Utimate Flea Trap (not shown), which further suggest that thermal cues have a minor role in host seeking.

Manufacturer specifications of all traps state their efficiency is much greater at night than during the day. Indeed, laboratory tests have shown that there is a significant difference between numbers of fleas collected when trapped for a consecutive 24 hour period compared with daylight only (Fig. 4). All traps performed significantly better when operated for 24 hours, although at different capacities. The three continuous light traps showed a 4 fold rise in trapping efficiency, although this is attributed to their poor performance, catching a relatively low number of fleas in both trials, which gives the larger apparent value. My Flea trap showed relatively good results when used only during the daylight hours, catching most of the released fleas (71.3 ± 4.3%), and almost all of the remaining ones in the 24 hour trials (92.6 ± 1.9%). These results clearly show that use of a light source as an attracting stimulus during the day can dramatically decrease trapping efficiency, unless a favorable light source with proper intensity and spectrum is used.

The fact that My Flea trap managed to catch most of the fleas during the daylight hour trials is encouraging as field sample collection for future research studies can be performed at any time, regardless of the competition from ambient light.

It must be kept in mind that Dryden and Broce (1993) speculated that the high efficacy of the Intermittent Light Trap could be explained by behavioral components of newly emerged fleas in search for a host. Newly emerged fleas perch on prominent objects in their larval habitat where they orient their bodies toward a light source or toward lighted areas. A sudden decrease of the light reaching the flea eyes is interpreted as a shadow created by a passing potential host. Thus, the green filter in the Intermittent

Light Trap provides a light source highly attractive to fleas whereas the light intermittence (ON – OFF) produces a stimulus that elicits flea jumping at the creation of a shadow.

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Figure legends

Figure 1: Traps used for urban and rural comparison trials: (A) My Flea Trap (Westham innovations) (B) The Ultimate Flea Trap (Victor®) (C) Springstar(D) Flea Beacon (Happy Jack inc.). Standard CD used for scale (12 cm diameter).

Figure 2: Trap comparison in rural field trials. Traps were set at four corners of site I, and rotated every 24 hours for 8 days (2 days at each corner for all traps). Shown is the average number of fleas caught in eight repetitions, each repetition representing 24 hours in which the trap occupied one corner of the stable (±SE). Statistical differences or similarities are signified by capital letters (ANOVA, Tukey)

Figure 3: Trap comparison in urban field trials. Traps were set at four corners of site II, and rotated every 24 hours for 4 days until all traps occupied all corners of the room. The trials were repeated in four different apartments. Shown is the average number of fleas (±SE) caught in eight repetitions, each repetition representing 24 hours in which the trap occupied one corner of the room. Statistical differences or similarities are signified by capital letters (ANOVA, Tukey Test)

Figure 4: Laboratory trap comparison trials. Shown are average of eight trials in which 100 fleas were released into a room with one of the traps positioned at the opposite corner, with fleas collected every 24 hours (black bars), or only during the daylight hours (gray bars). Capital letters signify statistical difference or similarities between traps (ANOVA, Tukey)

Figure 5: Comparison of modified ILT with CLT. Modified ILT traps were compared to the best performing CLT (Ultimate flea trap). SF – Synchronous light flickering. CL- continuous light (No flickering). AF – Alternating flickering. Statistical differences or similarities are signified by capital letters (ANOVA, Tukey)

Figure 6: Effects of modification of light spectrum: the light source of an ultimate flea trap was modified by placing a green filter over the light source, and trapping efficiency was compared to an unmodified trap. Results are average of nine repetitions (± SE).

Figure 7: Effects of heat on trapping efficiency: My Flea Trap was fitted with a heat source which was used with the light cue, or as the only attracting cue, and compared to My Flea Trap with no heat source and with no attracting stimuli. Shown is the average of fleas caught (± SE).

Figures:

Figure 1

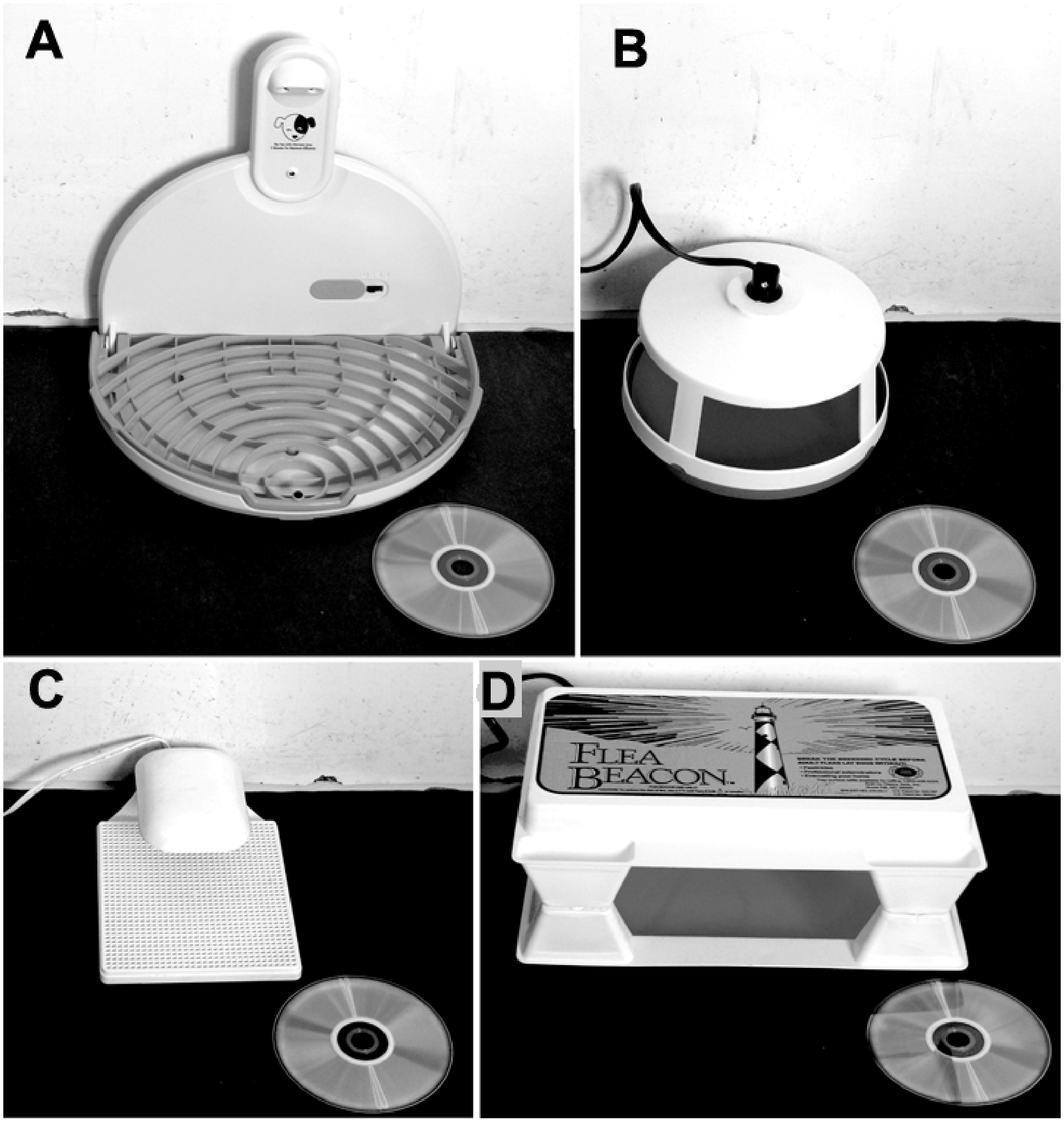


Figure 2:

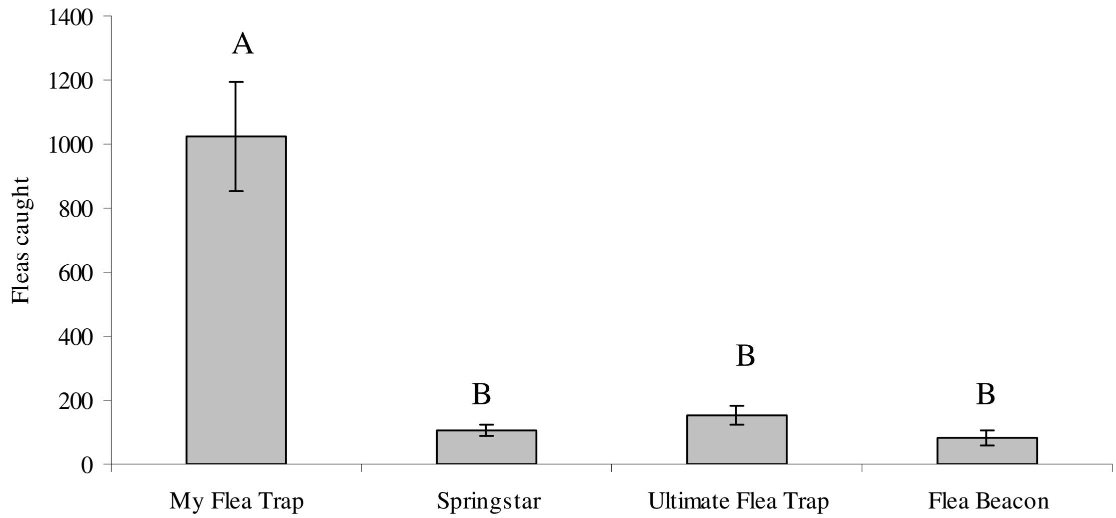
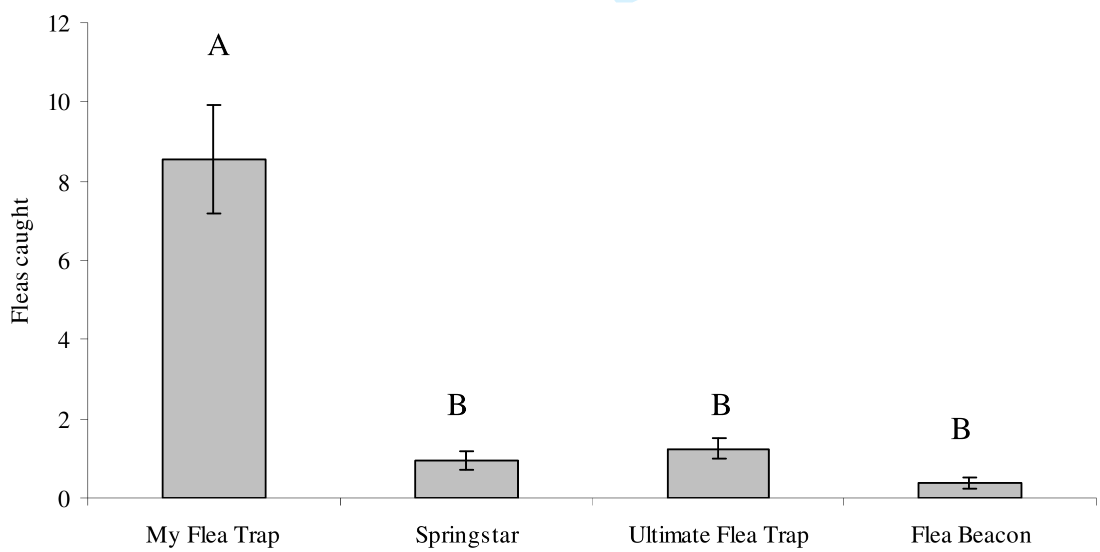


Figure 3



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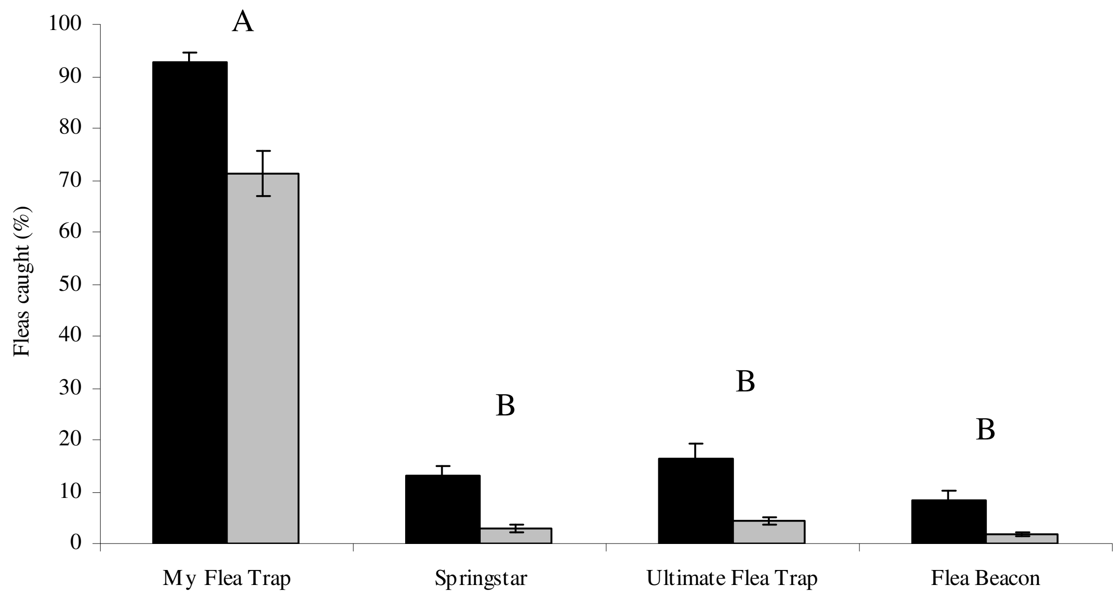
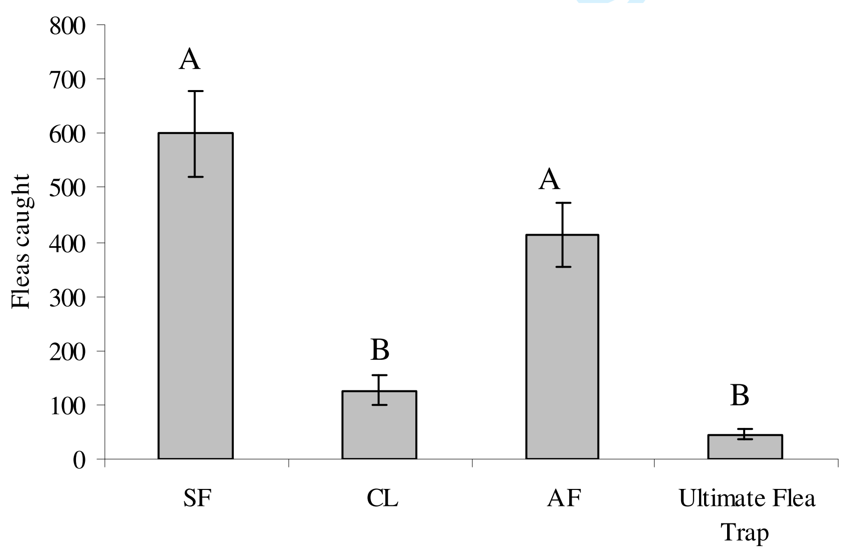


Figure 5



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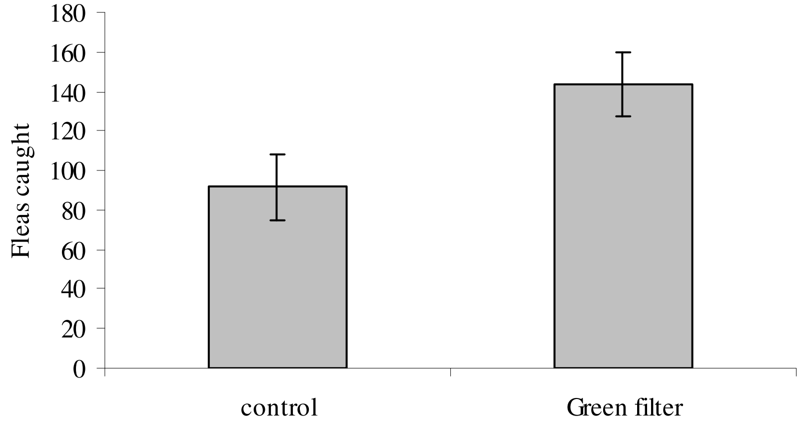
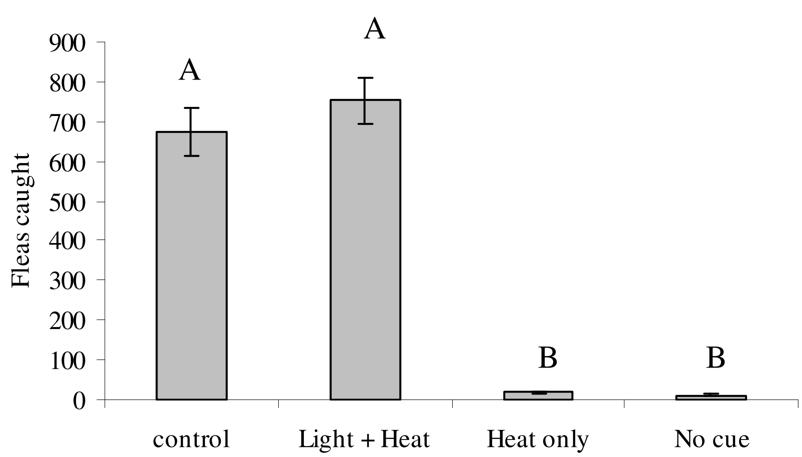
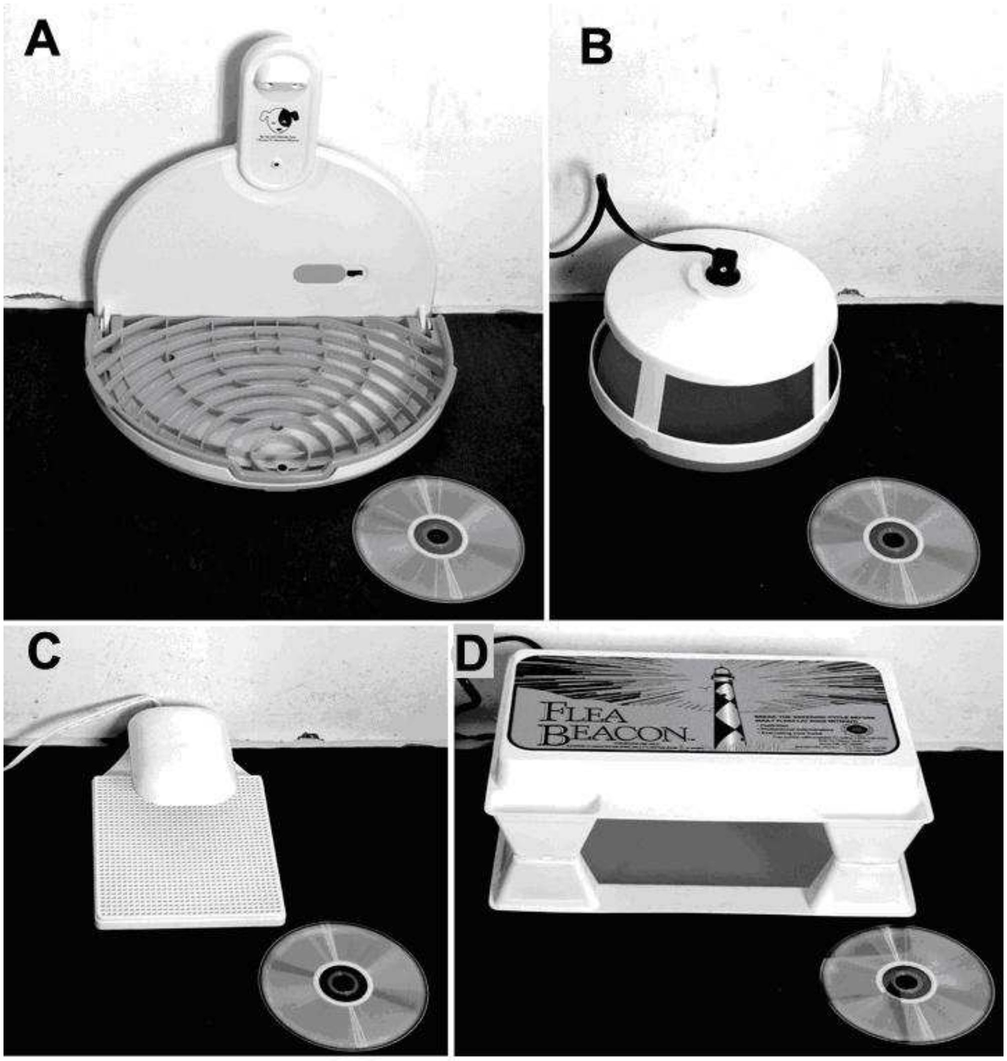


Figure 7





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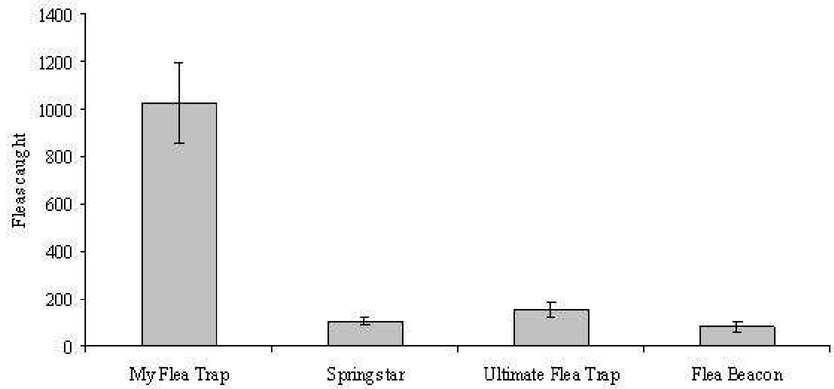

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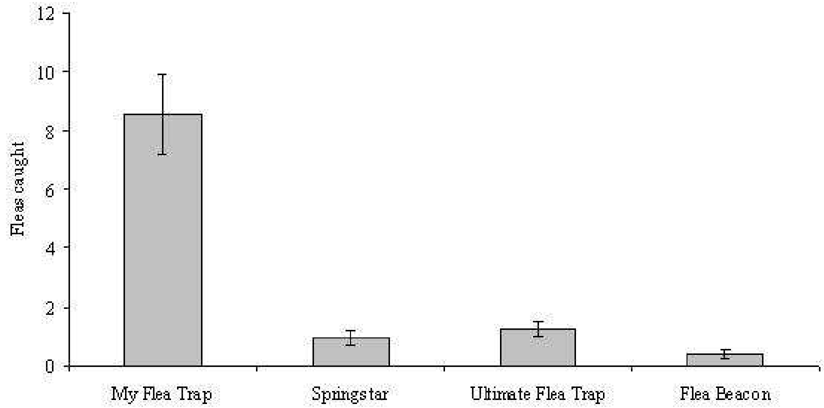

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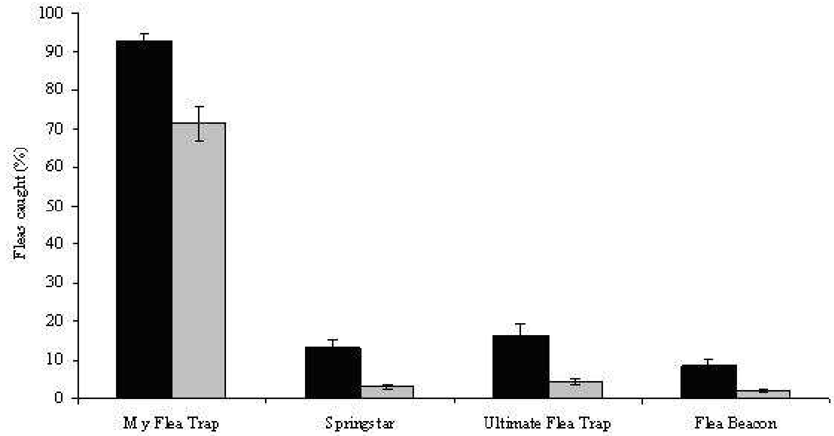

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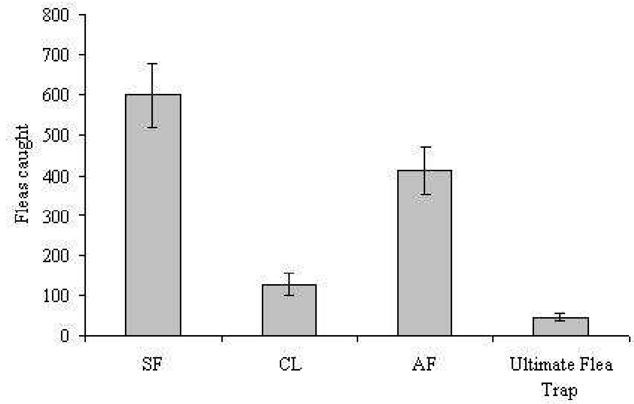

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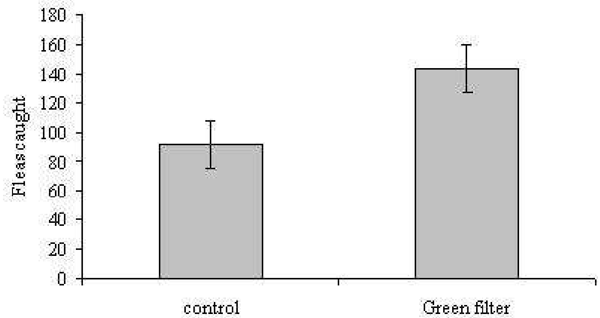

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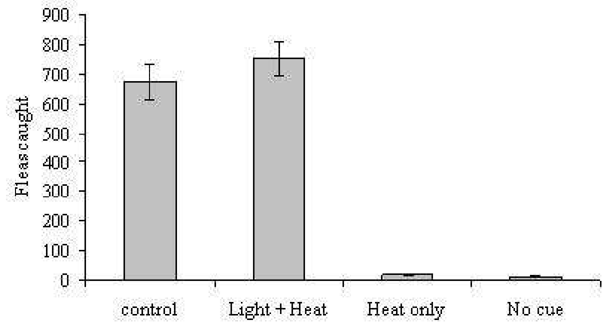

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